

Utilization of Microsatellite Markers to Test Purity and Hybridity of Canaryseed Genotypes



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16th March, 2015

Photo: <https://homepaddock.wordpress.com/2009/12/20/>

Out Line

- **Background**
- **Hybridity Test for F2 Families**
- **Purity Test in Seed lots**
- **Conclusion**



Photo: <http://www.bavisterspetcentre.com/canary-seed-1146-p.asp>

Background

Canaryseed (*Phalaris canariensis* L.),
annual canarygrass,
family (Graminaceae).
Self pollinated diploid ($2n = 12$).

**Canada is the largest producer
(64%) and exporter of canaryseed**

**over 90 per cent of production
grown in Saskatchewan.**



Photo: <http://www.fao.org/docrep/008/y5831e/y5831e06.htm>

Source: FAOSTAT 2013 & Saskatchewan Government, 2013

Not just for the birds

- ❑ Currently, canary seed has one end use, mixed birdseed,
- ❑ Promising cereal for human consumption and industrial applications.
 - ✓ Canary seed is a gluten free
 - ✓ Higher protein content, rich in tryptophan, cystine, and phenylalanine.
 - ✓ Five times more lipid than wheat,
 - ✓ Size of starch granules (1.5-3.5 μm)

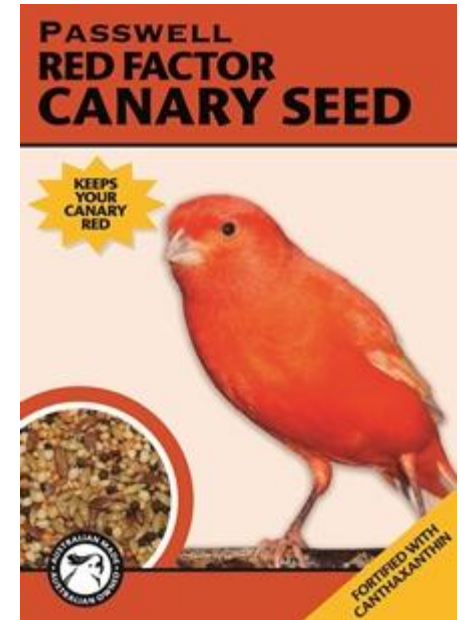


Photo: <http://www.birdandreptileproducts.com.au/bird-and-reptile-products/wombaroo-passwell/bird-products/red-factor-canary-s>

Ref. Abdel-Aal, J Agric Food Chem. 1997

Ref. www.canaryseed.ca/documents/Canaryseed_News_Fall_2011

- Siliceous hairs are highly irritating human skin or lungs
- CDC developed hairless (glabrous) varieties CDC Maria, CDC Togo and CDC Bastia.



Photo: <http://www.oardc.ohio-state.edu/weedguide/singlerecord.asp?id=50>

Hybridity Test in F₂ Families of Canaryseed.

Conventional breeding

To ensure hybridity of the progenies

- CGMS
- Emasculation



Photo source: <http://www.jugend.co.il/gallery/showphoto.php?photo=133005>

Molecular breeding

- Use of molecular markers
- Finding male parent-specific marker
- Progeny with such marker is a hybrid

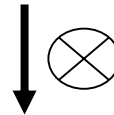
Materials and Methods

CDC USASK

♀ **C05091** **X** ♂ **C00038**



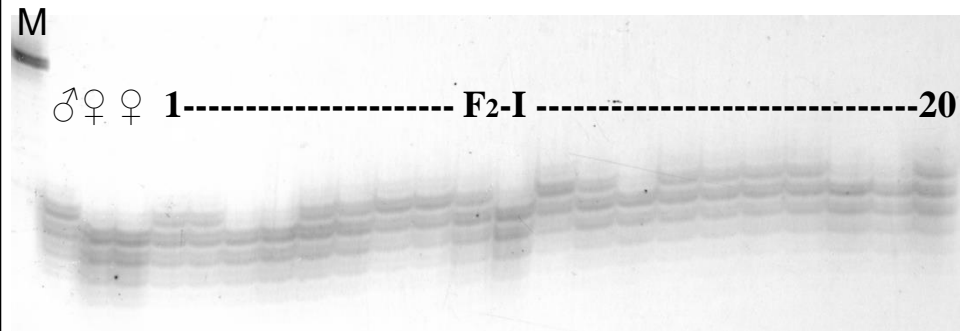
F1



F2 Families (11)

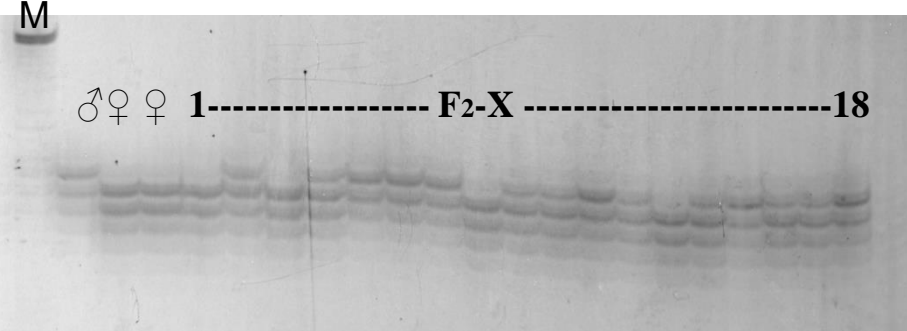
15 SSR primers of relatively high PIC (Li *et al.*, Mol. Breeding, 2011)

Marker CSM 39 with 20 individuals of F₂-I



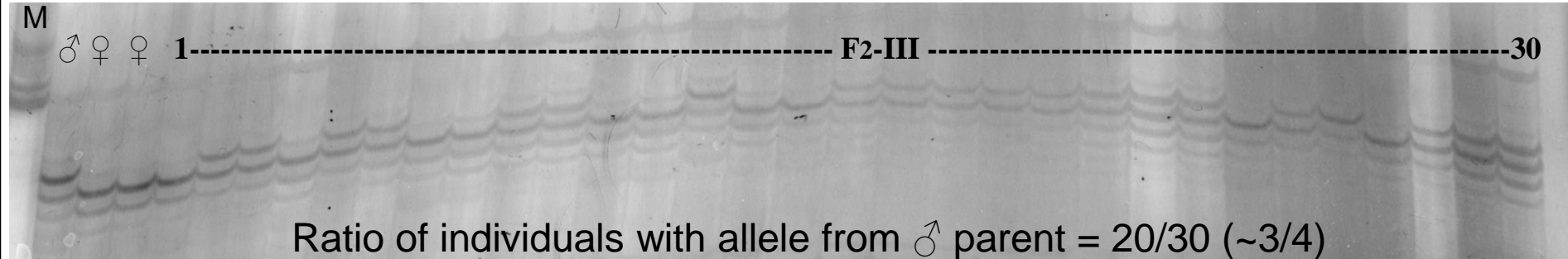
Individuals with allele from ♂ parent
14/20 ($\sim 3/4$)

Marker CSM 39 with 18 individuals of F₂-X



Individuals with allele from ♂ parent
14/18 ($\sim 3/4$)

Marker CSM 8 with 30 individuals of F₂-III



Ratio of individuals with allele from ♂ parent = 20/30 ($\sim 3/4$)

Conclusion Hybridity Confirmation of F₂ Families

Family	Marker	Family Size	Individuals Showed Male Parent allele	Hybridity
F2-I	CSM39	20	14	Yes
F2-II	CSM39	29	23	Yes
F2-III	CSM39	30	0	Yes
	CSM8	30	20	
F2-IV	CSM39	16	12	Yes
F2-V	CSM39	15	11	Yes
F2-VI	CSM39	7	6	Yes
F2-VII	CSM39	26	0	No
	CSM8	26	0	
F2-VIII	CSM39	27	27	Yes
	CSM8	27	27	
F2-IX	CSM39	42	32	Yes
F2-X	CSM39	18	14	Yes
F2-XI	CSM39	33	0	Yes
	CSM8	33	28	

Purity Test in Canary Seeds.

- ❑ Hybrid seed contamination during multiplication
 - Pollen shedders & out crossing
 - Physical admixtures
 - **Consequences:** segregation of the traits
- ❑ **Genetic purity test traditionally,**
 - Grow-out test (GOT)
 - Morphological traits
- ❑ **Molecular breeding**
 - Use of molecular markers
 - Finding line-specific marker
 - Seed lot with foreign line-specific marker



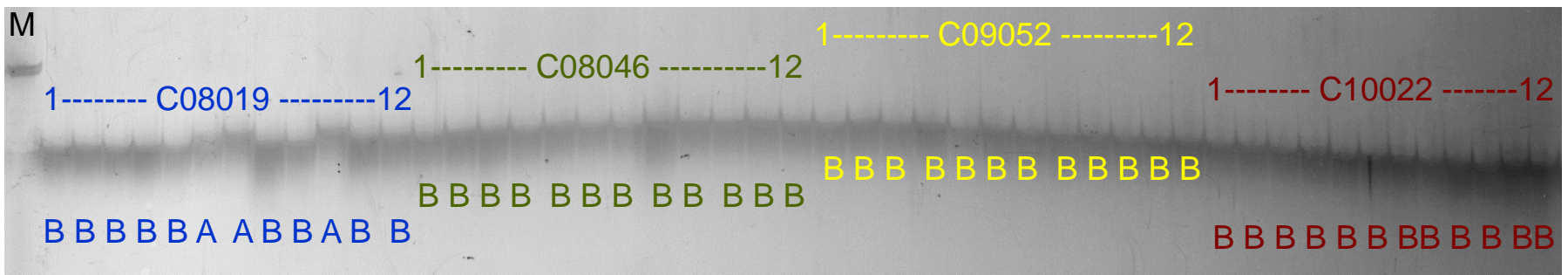
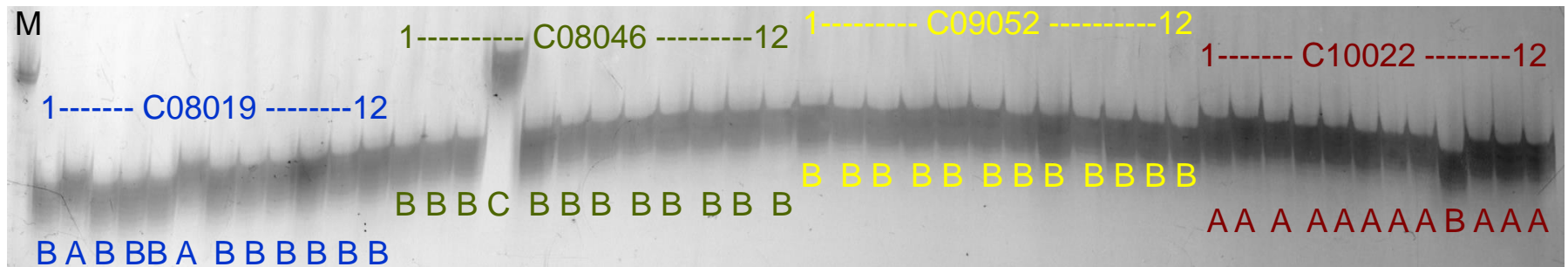
Photo: <http://dir.indiamart.com/impcat/canary-seed.html>

Materials and Methods

- CDC planted four canary seed lines C08019, C08046, C09052 and C10022 in four plots each A, B, C & D.
- Leftover seeds for each line in the CDC storage, control
- 15 SSR primers of relatively high PIC
(Li *et al.*, Mol. Breeding, 2011)

Seed Purity Test

- CSM58 with 12 plants
- a) Controls, b) Tested seed lot



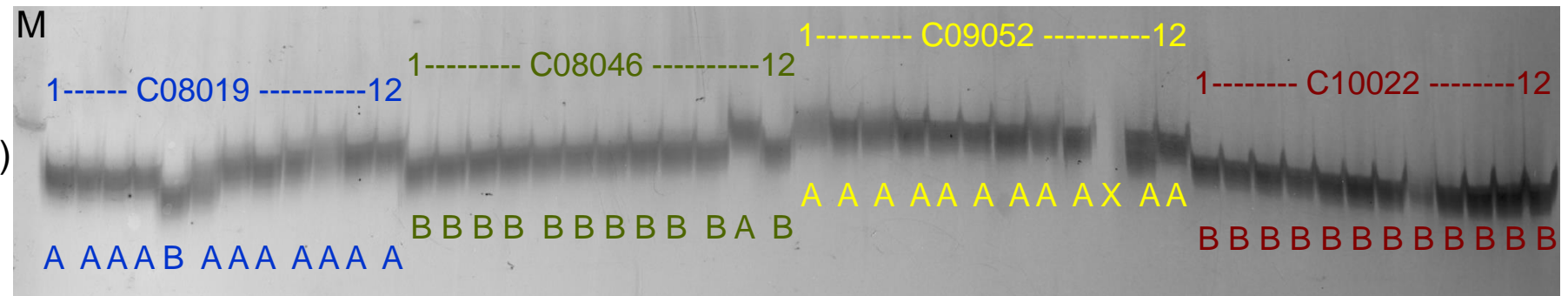
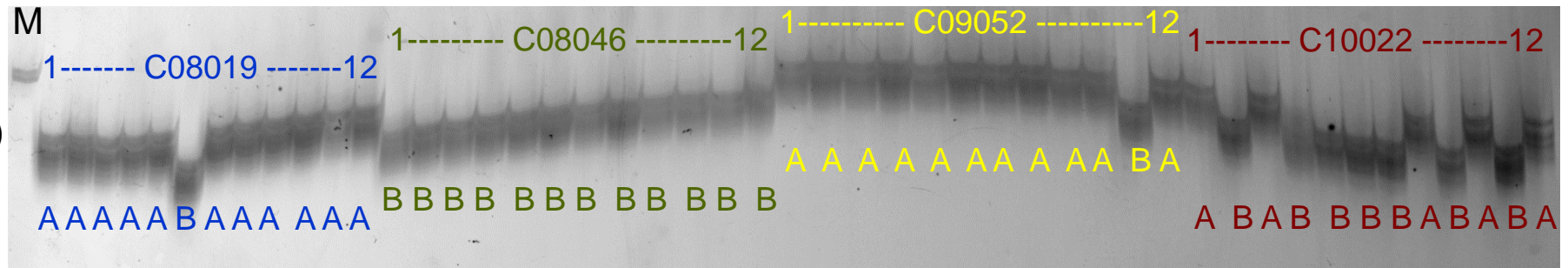
- CSM102 with 12 plants
- a) Controls, b) Tested seed lot

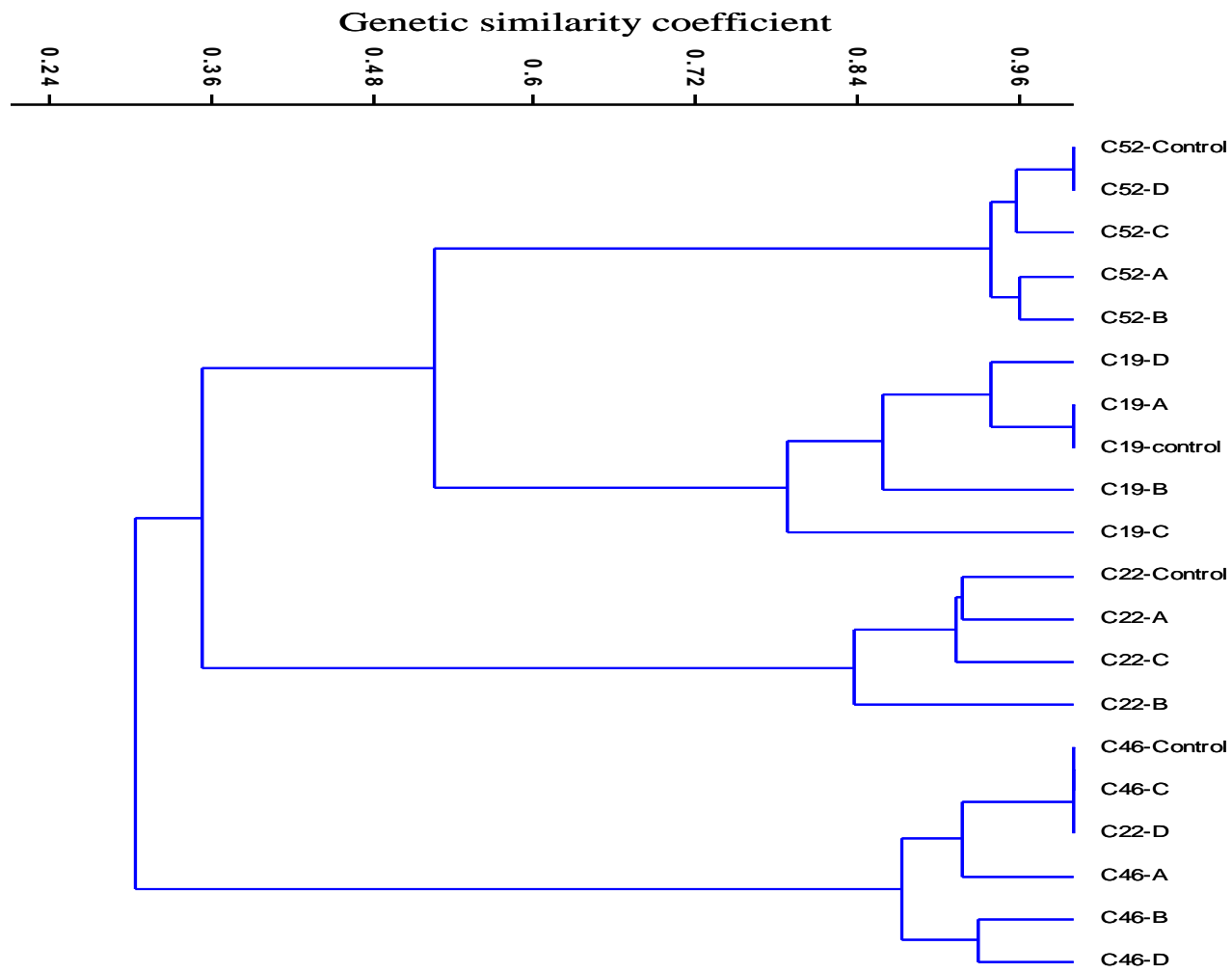
- CSM102 with 12 plants
- a) Controls, b) Tested seed lot



Seed Purity Test

- CSM85 with 12 plants
- a) Controls, b) Tested seed lot





Conclusion

➤ Validation of SSRs for hybridity & purity test

- ✓ 2 SSRs, 10 of F2 families to be true hybrids
- ✓ 3 SSRs, physical admixture in 1 out of 16 seed lots

➤ Possible outcomes

- ✓ Marker assisted breeding

➤ Next step

- ✓ Linkage between molecular markers and quality traits
- ✓ MAS

Acknowledgment

Dr. Ravindra Chibbar
Dr. Pierre Hucl
Dr. Monica Båga
And Technicians
Plant Sciences
University of Saskatchewan



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